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BRIEF REPORT

# Co-contamination of food products from family farms in an environmental disaster area in Southeast Brazil with pathogenic bacteria and enteric viruses

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## Abstract

In the present study, we evaluated the degree of contamination of fresh vegetables, cheeses and jellies from disaster area in Brazil with bacteria and enteric viruses. Food samples (n = 350) were tested for *Escherichia coli*, *Salmonella* spp., *Listeria monocytogenes*, *Staphylococcus* spp., and enteric viruses (rotavirus A (RVA), human adenovirus (HAdV), hepatitis A virus (HAV), and human norovirus (HNoV). *E. coli* was present in 56% of the samples, *Salmonella* spp. was present in 14% of the samples, *L. monocytogenes* and *Staphylococcus* spp. (coagulase-positive) were present in 36% of the samples. The enteric viruses RVA and HAdV were detected in cheeses and vegetables.

The World Health Organization (WHO) has reported 600 million episodes of illness in the world caused by contaminated food. These cases are often due to poor hygiene during the manipulation of fresh and processed consumer products [20].

Food contamination can occur at any stage of cultivation or processing by diverse routes, such as the application of animal waste to the soil as a fertilizer, contamination of water used for irrigation, direct contamination by livestock and wild animals, and contamination during processing of food products [14]. Poor sanitation conditions can result in the introduction of, pathogenic microorganisms to the water and soil that can cause foodborne diseases [16], the most common of which are caused by bacteria and viruses.

Enteric pathogenic bacteria, such as pathogenic serotypes of *Escherichia coli* (*E. coli*) and *Salmonella* ssp., can cause urinary infections, meningitis, diarrhea and other diseases. Bacterial diarrhea is one of the leading causes of death in children under five years old, especially in developing countries, accounting for 800,000 deaths per year around the world [4, 15]. Enteric viruses such as human adenovirus (HAdV), rotavirus A (RVA), human norovirus (HNoV), and hepatitis A virus (HAV) are potential bioindicators of food health security [5]. Despite the great importance of enteric viruses, testing for their presence in drinking water and food is not required in Brazil [8]. However, in developed countries, it is recommended to test water and raw food periodically, using HAdV is a bio-indicator of water quality. This virus is included in the List of Pollutant Candidates 4-CCL (CCL4) from the Environmental Protection Agency [19].

The city of Mariana, located in Minas Gerais State, Brazil, was affected in 2015 by an environmental disaster when the rupture of an ore dam negatively affected water, soil and air quality. The disaster changed the environmental landscape, destroying houses and agriculture fields. The most important economic activity in Mariana is family farming, where fresh food, including vegetables, cheeses, and jellies are produced for consumption in Brazil and other countries. This area has poor sanitation conditions, and a flow of mud caused human sewage and animal waste to be carried into water and soil used for food production [11].

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In this context, the present study aimed to identify the bacteria and enteric viruses present in foods produced, processed, and sold in the environmental disaster areas in Southeast Brazil (Minas Gerais State).

Foods produced and sold in the environmental disaster area in Southeast Brazil (Minas Gerais State) were collected from markets on three occasions during a two-month period. A total of 350 samples were collected. These samples were segregated into three groups: group 1, Minas Frescal cheeses (sample pool, n = 11); group 2, handmade jellies (sample pool, n = 8); group 3, vegetables (sample pool, n = 31, including spring onions, cucumbers and tomatoes (vegetables and fruits), and lettuce and arugula.

For isolation and identification of enterobacteria, duplicate 25-g portions of each food sample were collected from different points in the food, in duplicate.

The samples were enriched in 225 mL of 1% peptone-saline solution, incubated at 37 °C for 18 hours, and this homogenate was used to prepare dilutions of $10^{-1}$ to $10^{-3}$. After this period, *S. aureus; L. monocytogenes* (hemolytic), *E. coli* and *Salmonella* spp were identified using a series of biochemical tests (glucose fermentation, lactose fermentation, urea degradation, citrate degradation, motility, indole and $H_2S$ production) as described by the Food and Drug Administration [7].

Enteric viruses were isolated from foods using a buffer containing glycine and polyethylene glycol [9]. The viral genomic material in the samples was then extracted from 200 µL of concentrated sample using the commercial RTP Virus® Mini Virus Kit II (Invitek, Germany) according to the manufacturer's instructions.

Identification and quantification of HAdV, RV-A, HAV and HNoV was performed by real-time PCR (qPCR) using a TaqMan Universal PCR Master Mix Kit (Applied Biosystems, USA) and specific primers and probes, as described by Hernroth et al. [10], Zeng et al. [21], Jothikumar et al. [12] and Baert et al. [2], respectively. Mengovirus (vMCo) was used as an internal control of all virus analysis as described by Costafreda et al. [6].

*E. coli* was detected in 56% of the representative samples (28/50), *Salmonella* spp. was detected in 14% (7/50), and *S. aureus* (coagulase$^+$) and *L. monocytogenes* (hemolytic) were each detected in 36% (4/11) (Fig. 1).

In group 1 (composed of cheeses), there was a higher frequency of contamination by *E. coli* and RV-A than in group 2 (jams) and group 3 (vegetables). According to Amorim et al. [1], homemade cheeses are often sold in open markets or by street vendors, without due care at the time of manufacture and marketing. The results found in the present study corroborate the data of Nunes et al. [17], who, in a similar study, found that 13% of milk-derived samples were in poor condition due to the large number of coliforms, most of them fresh cheese in the Federal District (Brazil).

In group 3 (vegetables), enteric bacteria were more abundant than in group 1 (cheese). Baert et al. [2] argue that fruits and vegetables can be contaminated at harvest time because they are harvested by hand, and a lack of hygiene can result in contamination. These results corroborate a study by Kingsley [13], who reported outbreaks of hepatitis A associated with green onions imported to the US.

As shown in Figure 2, RV-A was more frequently found in food than HAdV, and HAV and HNoV were not detected (technique sensitivity limit ≥ 10 GC/g). Leafy vegetables such as cucumbers exhibited co-contamination by RV-A and HAdV (mean, 3Log10/g), indicating fecal contamination.

RV-A was more common in samples of different foods and was found in more than 50% of the samples evaluated, including leafy vegetables and cheeses. It can be inferred, due to the absence of HAdV, that this contamination came from animal feces. It should be noted that with the advent

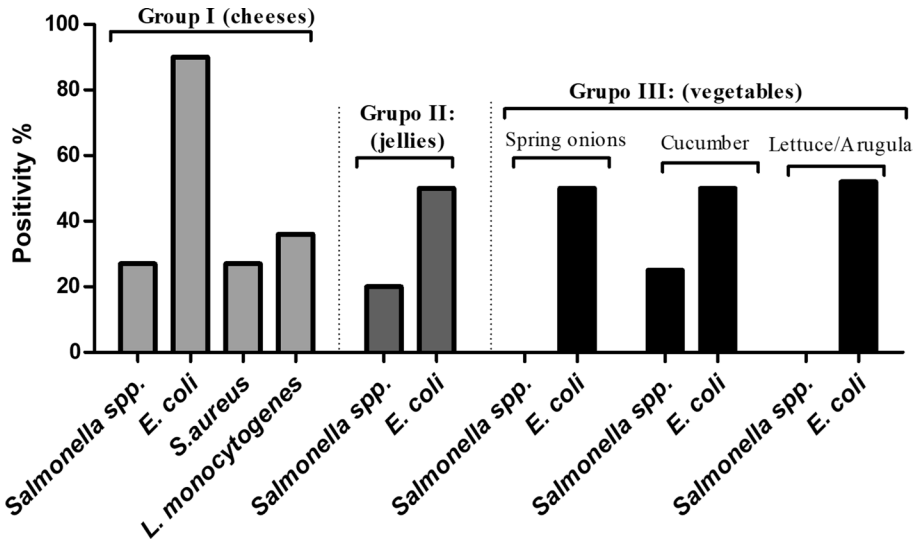


**Fig. 1** Positivity number of *Salmonella*, *E. coli*, *S. aureus* and *L. monocytogenes* in cheeses, jellies and vegetables from free faire

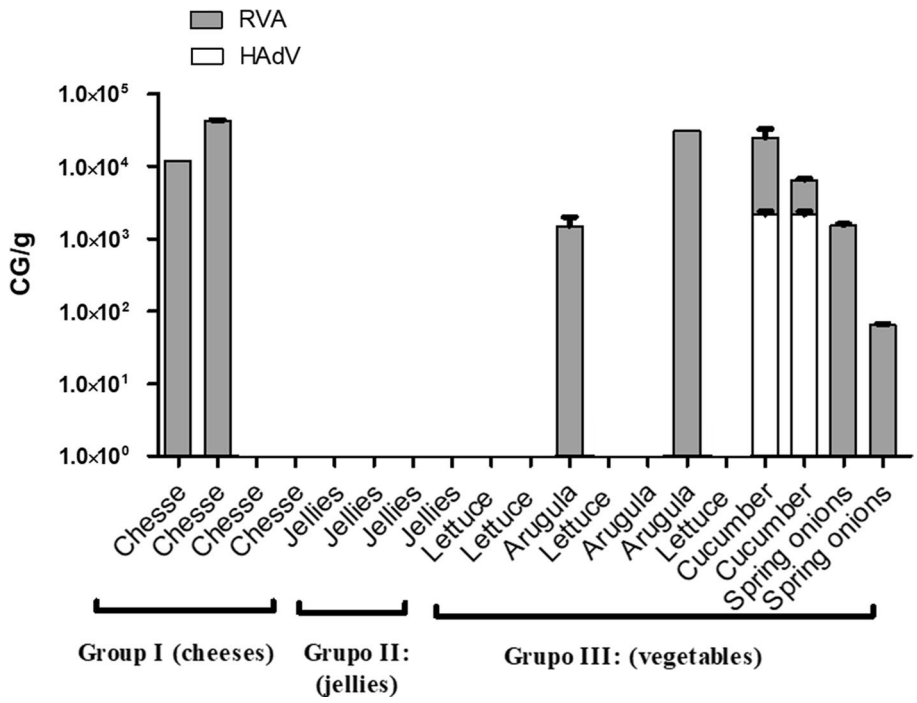


**Fig. 2** Average number of HAdV and RV-A in cheeses, jellies and vegetables from free faire

of vaccines, HAV is becoming increasingly rare in the developed world, while it remains endemic in developing countries, including Brazil [8].

The finding of enteric virus contamination in vegetables in this study is worrisome, since vegetables and—in particular, raw vegetables—are increasingly acting as vehicles of transmission of human pathogens, mainly viruses [3]. It is noteworthy that numerous outbreaks of foodborne viruses have been associated with the consumption of contaminated fresh produce [14, 18].

These results reinforce the need for monitoring and sanitary control of pathogens, that cause outbreaks of food-related gastroenteritis and this should be included in national legislation. In this sense, this study may help in future decision-making and establishment of techniques and training of inspectors testing for foodborne pathogens in the state of Minas Gerais in order to meet international standards for microbiological control and investigation of viral outbreaks, particularly those of gastroenteric pathogens, since this is a requirement for food exports to several countries, such as European countries.

Co-contamination with pathogenic bacteria and enteric viruses was observed in foods from family farms sold within an environmental disaster area in Brazil. We expected that this study will help in the management of microbiological risks in fresh food and minimally processed foods produced in rural areas and will have a positive impact on family agriculture and human nutrition.

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